

First Case of Disseminated Infection with *Nocardia cerradoensis* in a Human

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Here we report in a human, a renal transplant patient, the first disseminated infection with *Nocardia cerradoensis*, isolated after a brain biopsy. Species identification was based on 16S rRNA, *gyrB*, and *hsp65* gene analyses. Antibiotic treatment was successful by combining carbapenems and aminoglycosides and then switching to oral trimethoprim-sulfamethoxazole.

CASE REPORT

A 59-year-old woman with a history of end-stage renal disease, secondary to autosomal-dominant polycystic kidney disease, received a renal transplant in 2010. Her immunosuppressive regimen included tacrolimus at 12 mg/day and prednisolone at 7.5 mg/day. Prophylaxis with trimethoprim-sulfamethoxazole (SXT) was given for 6 months after the transplantation and then stopped due to intolerance symptoms. The patient had no history of recent travel. Two weeks before admission, she had had a mild headache with fever and chills and she was empirically treated with amoxicillin and prednisone for 7 days. After a brief improvement, she developed a nonproductive cough with dyspnea. Based on the hypothesis of pneumonia, antibiotic treatment was switched to ceftriaxone combined with aerosol bronchodilators. The patient's pulmonary symptoms rapidly worsened, and in September 2013, she was admitted to the hospital for further investigation.

On examination, the patient was awake and complained of inspiratory dyspnea. Diarrhea was noted a few days after the initiation of antibiotic treatment and resolved spontaneously. The patient's temperature was measured at 37.8°C, blood pressure was 150/90 mm Hg, pulse was 97 beats/min, respiratory rate was 36/min, and oxygen saturation was 97% while she was breathing ambient air. The auscultation evidenced bilateral basal crepitus and no other abnormal sounds. Heart sounds were normal, abdomen was soft, without tenderness, distention, or organomegaly, and neurologic examination was normal. No peripheral lymph nodes were detected. Subcutaneous nodules of the lower extremities appeared a few days after the patient was admitted (Fig. 1A). The white cell count was 18.6×10^9 /liter, with 95% neutrophils, and the blood level of C-reactive protein was 159 mg/liter. A chest X-ray and a computed tomography (CT) scan showed wall thickening of the right main bronchus, moderate ground-glass opacities of the right upper lobe, a right hilar lymph node, a small right parenchymal nodule (<10 mm), and moderate homolateral pleural effusion (Fig. 1B). An abdominal CT scan revealed diffuse peritoneal and right retroperitoneal nodules (Fig. 1C) and a thickening of the cecal wall. Bronchoalveolar lavage (BAL) fluid, bronchial, and subcutaneous biopsy specimens were collected, and examination of stained smears (using Gram, Ziehl-Neelsen, May-Grünwald-Giemsa, Papanicolaou, Perls, and hematoxylin-saffranin stains) showed no bacteria or abnormal cells. Cultures for bac-

teria, fungi, and mycobacteria were all negative. Histological examination of the subcutaneous nodule identified an erythema nodosum. Amplification of bacterial 16S rRNA gene from the cutaneous biopsy specimen was also negative. The diagnosis of primary lung carcinoma was then suggested, and a transbronchial right hilar lymph node puncture was performed and did not show any malignant cells; bacterial cultures were not done due to insufficient sampling.

On day 20 of hospitalization, the patient presented worsening neurological symptoms, with generalized weakness, dysarthria, lateral seizures, and pyramidal syndrome on examination. A brain CT scan, without the administration of contrast material, followed by magnetic resonance imaging (MRI) showed no hemorrhage of brain parenchyma and small nonspecific lesions suggesting a differential diagnosis of multiple abscesses or cystic or necrotic brain tumors (Fig. 1D). A stereotactic biopsy of brain parenchymal lesion was performed, and Gram staining showed filamentous Gram-positive bacilli (Fig. 1E). Bacterial growth was obtained on chocolate and buffered charcoal-yeast extract plates (oriented by Gram staining) incubated at 37°C for 48 h in an aerobic atmosphere; the 2-mm-diameter colonies were white and rough. A forehead skin swab collected during neurological surgery and two positive aerobic blood culture (Bactec) samples, both plated on chocolate agar, allowed after 48 h of incubation the isolation of a bacterium with the same morphological features as those described above. The cerebrospinal fluid remained sterile after 10 days of incubation.

Matrix-assisted laser desorption ionization–time-of-flight mass spectrometry (MALDI-TOF; Bruker, France), even after a

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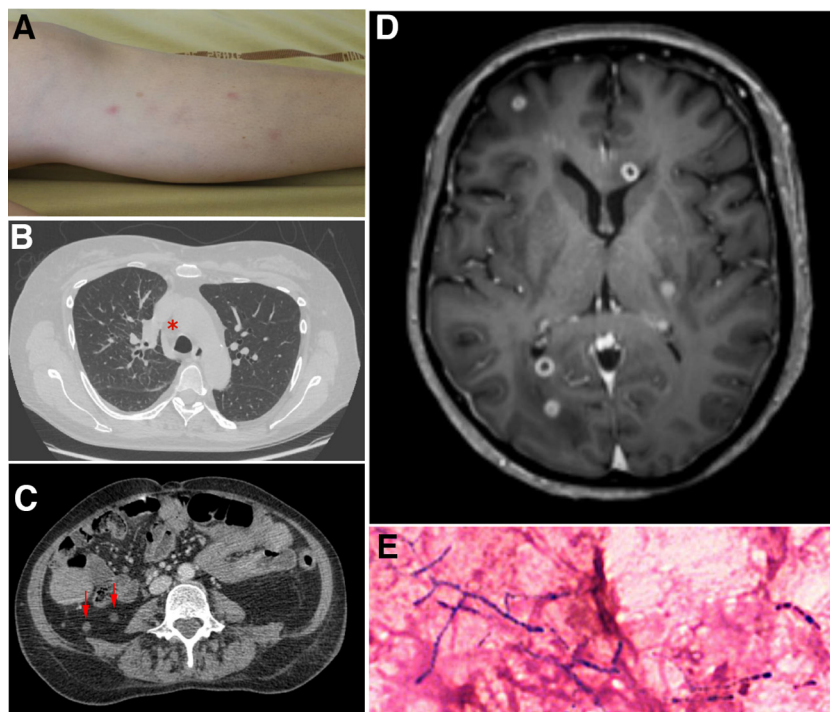


FIG 1 Disseminated nocardiosis. (A) Subcutaneous nodules of the lower extremities; (B) right hilar lymph node (indicated by asterisk); (C) retroperitoneal nodules (arrows); (D) multiple brain abscesses (MRI); (E) Gram stain (magnification, $\times 100$) of cerebral biopsy specimen.

full protein extraction, yielded identification of a *Nocardia* species with a score of <1.7 (in 2014, 5,627 species were included in the Bruker spectrometry database). To better identify all the isolated bacteria, we carried out the sequencing of 16S rRNA, *gyrB*, and *hsp65* genes (1), which were queried against the GenBank database. The highest nucleotide identities (%) for each gene were as follows: (i) 16S rRNA gene, 99.84% identity with *N. cerraadoensis* (2 different nucleotides [nt]/1,325-nt fragment), 99.62% with *Nocardia mikamii* (5/1,325 nt), 99.47% with *Nocardia africana* and *Nocardia aobensis* (7/1,325 nt), and 99.39% with *Nocardia veterana* (8/1,325 nt); (ii) *gyrB* gene, 98.98% identity with *N. cerraadoensis* (10/990 nt) and 98.28% with *N. mikamii* (17/990nt); (iii) *hsp65* gene, 99.76% identity with *N. cerraadoensis* (1/434 nt) and 98.84% with *Nocardia nova*, *N. africana*, and *N. aobensis* (5/434 nt). Therefore, the confirmed identification of the isolated strain from the patient's brain abscess, here named OFN13.186 (Observatoire Français des Nocardioses, Lyon, France), was *Nocardia cerraadoensis*.

The antimicrobial susceptibility of strain OFN13.186 was tested by using a broth microdilution method according to CLSI standard M24-A2 guidelines (2). The MICs were studied as previously described (1), by using Sensititre rapidly growing mycobacteria plate format (RAPMYCO) plates, incubated at 37°C for 72 h. Based on the previously established breakpoints for the *Nocardia* genus (1), the strain was resistant to amoxicillin-clavulanic acid, piperacillin-tazobactam, tobramycin, minocycline, and fluoroquinolones (Table 1). After consideration of the intolerance of the patient to SXT, empirical intravenous treatment with meropenem (4 weeks) and amikacin (2 weeks) was started, which allowed rapid clinical improvement and regression of all the neurological disorders. The patient was discharged, and after desensitization (3), antimicrobial treatment was switched to oral SXT for 6 weeks. Two months after the initiation of antimicrobial ther-

apy, the intraabdominal nodules and thoracic lymph nodes had completely regressed, but a lung parenchymal nodule (5 mm) persisted. A follow-up of the pulmonary nodule and long-term prophylaxis with SXT (800 mg trimethoprim/160 mg sulfamethoxazole) were then maintained.

The genus *Nocardia* comprises a ubiquitous group of environmental bacteria found in soil, water, or decaying organic matter,

TABLE 1 Antimicrobial susceptibility testing results for strain OFN13.186

Antibiotic(s)	Observed MIC ^a	Interpretation ^b
Amikacin	≤ 4	S
Amoxicillin	4	S ^c
Amoxicillin-clavulanic acid	$>32/16$	R
Cefotaxime	≤ 4	S
Ceftriaxone	≤ 4	S
Cefepime	≤ 4	S
Ciprofloxacin	>4	R
Clarithromycin	≤ 2	S
Imipenem	≤ 2	S
Linezolid	≤ 4	S
Minocycline	2	I
Moxifloxacin	2	I
Tobramycin	16	R
SXT	$\leq 1/19$	S

^a MICs were determined by the broth microdilution method (see reference 1 for breakpoints and methods). SXT, trimethoprim-sulfamethoxazole.

^b S, susceptible; R, resistant; I, intermediate.

^c Interpretative criteria used for amoxicillin were based on amoxicillin-clavulanic acid breakpoints (CLSI document M24).

and more than 50 species have already been described (4, 5). Opportunistic infections with *Nocardia* in humans occur mostly in patients with cell-mediated immunodeficiency, such as transplant recipients. Here, and as far we know for the first time, we report a human case of diffuse nocardiosis due to the species *Nocardia cerradoensis*, which was recently described as a new species from a strain isolated from Cerrado soil in Brazil in 1985, strain YT⁹ (6).

Immunosuppressive drugs required after kidney transplant induce immune cell deficiency, which is formally recognized as a risk factor for diffuse nocardiosis (7). However, nocardiosis remains a diagnostic challenge, because it has a rare etiology in comparison to diseases from other opportunistic pathogens, clinical presentation is nonspecific, and the bacteria are often difficult to culture. The most common route of entry of *Nocardia* spp. is inhalation or aspiration of the organism, as they can become airborne, particularly on dust particles (4, 5, 8). In this case, the initial foremost respiratory symptoms and the main involvement of the right upper lobe and hilar lymph node suggested that the respiratory tract was the primary site of infection. Although primary cutaneous infection or more occasionally infection spreading from the oral cavity or gastrointestinal tract may occur, we believe that the cutaneous nodules, which appeared after the onset of symptoms (Fig. 1), as well as cecal thickening and abdominal nodules were associated with disseminated disease. Additionally, cutaneous nodules mimicking erythema nodosum or cellulitis have been previously described in disseminated infections (9). Unlike other bacterial brain abscesses, neurological symptoms in cases of nocardiosis are highly variable, generally insidious, and without fever or signs of septicemia. The most common signs are focal neurological deficits, nonfocal findings, and seizures (10). In our patient, the acute CNS symptoms associated with multiple small cerebral abscesses were concomitant with a rapid dissemination of infection, since all the bacterial cultures became positive. However, the most likely scenario is that the brain metastases were present initially despite a normal neurological examination.

At the onset of the infection, a short course of antibacterial treatment (amoxicillin followed by ceftriaxone for 2 weeks) may explain the brief improvement in clinical status and negative bacterial cultures despite efforts to find nocardiosis. *Nocardia* species exhibit a species-predictable antimicrobial susceptibility pattern, and early species identification may be a crucial step to start an adapted antibiotherapy (11). When bacterial cultures were positive, the isolates were correctly identified by MALDI-TOF at the genus level, and the lack of accurate identification at the species level is easily explained because *N. cerradoensis* was not included in the database. Thus, the identification of *N. cerradoensis* was obtained by sequencing three housekeeping genes (16S rRNA gene, *gyrB*, and *hsp65*) as previously described (12, 13). The criterion used was that described by the CLSI MM18-A document, i.e., for identification at the species level regardless of the gene used, an identity higher than 99.6% is required (14).

In addition to activity against *Nocardia* species, the antibiotics considered should also exhibit good diffusion to all sites of infection. In our case, we decided to initiate treatment with wide-spectrum antibiotics that also cross the blood-brain barrier. Actually, *Nocardia* shows a lower percentage of resistance to linezolid, SXT, and amikacin (11, 15). Carbapenem usually displays a low MIC, and meropenem exhibits the best cerebral diffusion among this class. Time-kill studies showed that imipenem-amikacin and imipenem-moxifloxacin combinations are bactericidal for most iso-

lates, whereas linezolid and SXT exhibit mainly bacteriostatic activity (16). The recognized patient intolerance to SXT, and the limit of the initial identification to the genus level, did not allow us to make a decision for antibiotic treatment based on the *in vitro*-predicted susceptibility. Therefore, the first-line antibiotherapy combined meropenem and amikacin, to which the bacteria have subsequently been shown to be susceptible (Table 1). Antibiotic susceptibility test results and patient improvement led us to narrow the antibiotic spectrum and start treatment with SXT (800 mg trimethoprim/160 mg sulfamethoxazole) after a desensitization protocol (3). Prophylactic treatment with SXT was then instituted.

This is the first case of human disseminated infection with *N. cerradoensis* occurring in an immunosuppressed host. Nevertheless, even if infection with a *Nocardia* species was initially strongly suspected among other opportunistic pathogens, it is essential to make the precise diagnosis at the species level to institute the adapted antimicrobial regimen. Finally, because *Nocardia* species can invade the CNS silently (4), we would like to emphasize, as recommended by others (17), that an MRI of the brain should be systematically considered for diagnostic evaluation even in the absence of neurological symptoms.

Nucleotide sequence accession numbers. The sequences for the following *Nocardia cerradoensis* (strain OFN 13.186) genes have been deposited in GenBank under the indicated accession numbers: *gyrB*, accession no. KP013615; 16S rRNA gene, accession no. KP013616; *hsp65*, accession no. KP013617.

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